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DETERMINATION OF SUBPICOGRAM QUANTITIES OF DRUGS AND NARCOTICS IN URINE OF HUMAN SUBJECTS

Final Report

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April 1976

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The urinary excretions of methaqualone and 6-hydroxymethaqualone (free and conjugated) were studied in a normal male subject over a period of 30 days using stable isotope dilution analysis and field ionization mass spectrometry. The terminal half-lives of three compounds were estimated to be about 70 hr. These results were tentatively interpreted in terms of a multicomponent pharmacokinetic model. The ability of isotope dilution analysis using field ionization mass spectrometry to detect minute quantities of drugs in urine was thoroughly evaluated →

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by these experiments. The sensitivity limit was between 0.1 and 1 ng/ml of urine, and was primarily limited by the chemical purity of the isolated drug or metabolite.

CONTENTS

I INTRODUCTION

This is a final report on a research program sponsored by the Army Medical Research and Development Command under Contract #DADA17-73-C-3063.

The objective of this research program has been to determine very low concentrations of drugs and their metabolites in human urine by the use of nonradioactive isotope dilution analysis. By the use of multi-labeled organic molecules as isotopic dilutants and nonfragmenting field ionization mass spectrometry as the analytical instrument, subnanogram quantities of drugs can be quantitatively determined. This high sensitivity of detection may allow us to determine the rates of excretion of drugs and their metabolites over far longer time periods than ever before possible. Such data will not only provide a better understanding of long-term effects of drugs, but may also permit more effective drug control by the positive identification of drug abusers even after long periods of abstinence.

During the third year of this research program, we have applied the technique of isotope dilution analysis to the determination of methaqualone (MTQ) and 6-hydroxymethaqualone (MTQOH) in urine samples provided by the sponsor. The high sensitivity of this technique enabled us to determine that low levels of MTQ and MTQOH appear in urine for thirty days after ingestion of one tablet of MTQ.

Section II of this report contains a review of the work performed during the preceding two years and the first quarter of this year. This information appeared in a published article.[1] A description of the new methodology developed for the more rapid determination of MTQ and MTQOH in urines is presented in Section III. This methodology was applied to 58 urine samples collected after ingestion of a single tablet of MTQ, and the results are given in Section IV. Section V presents a preliminary pharmacokinetic interpretation of the results. Section VI contains the conclusions of our study.

II DETERMINATION OF PICOMOLE QUANTITIES OF METHAQUALONE AND 6-HYDROXYMETHAQUALONE IN URINE

Methaqualone [MTQ*; 2-methyl-3-o-tolyl-4(3H)quinazolinone] is a non-barbiturate sedative hypnotic used medically for the treatment of insomnia and anxiety. In recent years, MTQ abuse has increased to almost epidemic proportions among youthful drug users.[2-4] An historical review was recently published describing the rapid rise in MTQ abuse in several nations of the world, and presenting evidence that MTQ can induce physical dependency similar to the barbiturates.[5]

A number of analytical techniques have been devised for the identification of MTQ and its metabolites in plasma and urine samples. These include ultraviolet spectrophotometry,[6,7] fluorimetry,[8] thin-layer chromatography (tlc),[9,10] gas chromatography,[11-14] and gas chromatography-mass spectrometry.[15-18] Some of these methods have been used to obtain quantitative information on the bioavailability[19] and pharmacokinetics[11,17,18,20-22] of MTQ. Although studies carried out for only a few hours indicated rapid disappearance of MTQ from plasma ($t_{\frac{1}{2}}$ = 2.6 hr),[20] more extended investigations lasting for as long as 5 days have shown that the half-life of MTQ in plasma is, in fact, much longer (19.6 to 41.5 hr).[17] These reports are not necessarily inconsistent, since they probably reflect different rate-limiting physiological processes.

The aim of the studies reported here were: (1) to develop an ultra-sensitive analytical procedure for the analysis of MTQ and one of its metabolites, 6-hydroxymethaqualone (MTQOH), in urine, and (2) to apply this methodology to the analysis of urines collected over extended times following drug ingestion. The information obtained from such studies should lead to a more thorough understanding of the persistence of MTQ in humans and may also permit more effective control over nonmedical uses of MTQ.

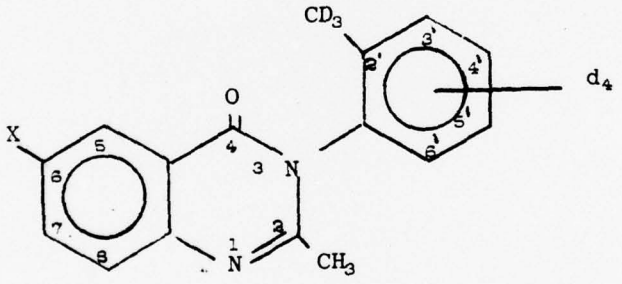
IIa EXPERIMENTAL

Chemicals

Solvents used in tlc were Eastman Spectro-Grade. Deionized water was distilled from alkaline permanganate before use. High purity silica gel (Silica Gel G-HR/UV-254; Macherey, Nagel & Co.), used in tlc, was further purified by washing in 80% (v/v) acetonitrile/water, chloroform, and methanol, and was oven-dried (100°C).

Synthesis of Multilabeled MTQ and MTQOH

Commercial d_8-toluene (Merck) was converted to o-toluidine-d_7 by nitration and reduction of the nitro group. The labeled toluidine was condensed with N-acetyl-anthranilic acid to produce heptalabeled MTQ (I).[23] The field ionization mass spectrum of the



I X = H

II X = OH

deuterated compound is shown in Figure 1, and the ratio of counts obtained at the masses of the unlabeled and fully labeled compounds is indicated.

Exchange of the 2-methyl protons of I was achieved by dissolution of the compound in CH_3OD and addition of a catalytic amount of CH_3ONa. The resulting MTQ (M + 10) was used for some analyses, but the added deuterons proved to be exchangeable under the conditions of tlc. Most of the experiments were therefore performed using the M + 7 compound.

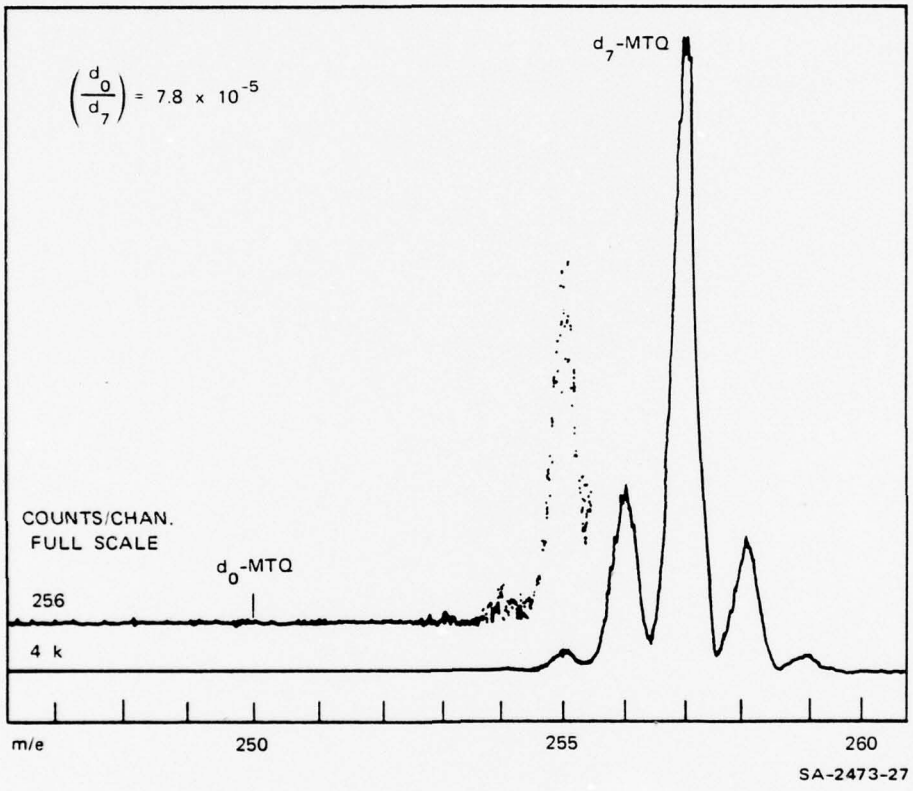


FIGURE 1 FIELD IONIZATION MASS SPECTRUM OF d_7-MTQ

Synthesis of M + 7 MTQOH (II) was achieved in a similar fashion to that of MTQ, by condensation of 5-methoxy-anthranilic acid with the d_7-o-toluidine, followed by demethylation. The field ionization mass spectrum of the heptalabeled MTQOH is shown in Figure 2. The details of these syntheses and the characterizations of the compounds will be described elsewhere.[24]

Instrumentation

Molecular ions are produced using a multipoint field ionization source operating at an ionizing potential of 2 kV.[25] To record the ratio of unlabeled to labeled MTQ or MTQOH, the molecular ion region is scanned repetitively by varying the accelerating voltage, and the multiplier output is integrated by a multichannel analyzer. The analyzer sweep controls the scan via a programmable power supply. A retarding lens on the detector is operated at close to source potential to increase the abundance sensitivity and to reduce the scattered ion background. Samples are introduced into the spectrometer with a solid inlet probe, and data are recorded over the temperature range 60° - 100°C for MTQ and 100° - 125° for MTQOH.

Extraction of MTQ and MTQOH from Urine

To a 5 ml urine sample was added 1 drop of glacial acetic acid, 0.5 ml of 1 M acetate buffer (pH 5), and approximately 0.07 I.U/ml of β-glucuronidase and 0.035 I.U/ml of aryl sulfatase (Calbiochem B grade). The sample was incubated at 37°C for 18.5 hr, then was approximately neutralized by adding 2 drops of 10 M NaOH and 0.6 ml of 1.5 M potassium phosphate, pH 6.8. A similar urine sample, not hydrolyzed, was used for the determination of unconjugated MTQOH.

Multilabeled MTQ (M + 7 or M + 10) and MTQOH (M + 7) were added to the urine samples from stock aqueous solutions whose concentrations were determined spectrophotometrically.[26] The amount of labeled compound added

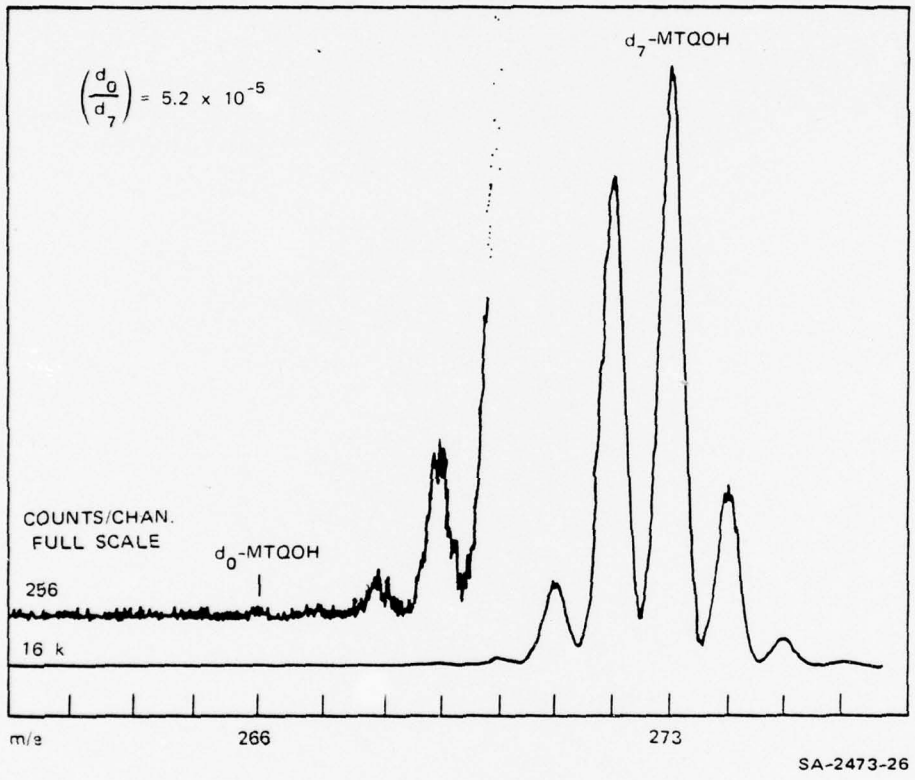


FIGURE 2 FIELD IONIZATION MASS SPECTRUM OF d_7-MTQOH

was typically 15-30 μg. Hydrophobic substances were then extracted from the samples by passage through small polypropylene columns (Bio-Rad 731-1110) whose lower portions were filled with washed Amberlite XAD-2 resin.[27]

The extraction procedure involved, initially, filtration of the urine samples through Whatman No. 1 paper directly onto the columns. Following two 10 ml washings with water and expulsion of residual water with nitrogen, the bulk of the lipophilic materials was eluted in two 10 ml washings with ethyl acetate/chloroform (2/3; v/v). The eluates were dried with Na_2SO_4 and concentrated under a nitrogen stream. Between sample applications, the columns were washed free of residual pigments according to a published procedure.[28]

Purification of MTQ and MTQOH by tlc

MTQ and MTQOH were isolated from the concentrated eluates by four chromatographies on silica gel plates. The initial purification step involved a bidimensional chromatography. Following this step, the ratios of counts observed at the major masses of the unlabeled and labeled compounds is about five times higher than background, using control urine samples. This ratio is adequate for determining the two compounds at concentrations greater than 5 ng/ml. It will be seen in the next section that the concentration of MTQ in urine decreases to this value after approximately 7-9 days. Determination of MTQ at longer times requires a more thorough purification. A second bidimensional chromatography reduces the observed ratio to the background level. The chromatographic systems used and the observed R_f values are given in Table I.

It should be emphasized that the appaication of multiple tlc to hydroxylated MTQ derivatives most probably results in the exclusive isolation of the 6-hydroxy isomer. It is known that at least three of the solvent systems used here discriminate strongly among the hydroxylated MTQ derivatives.[10,29] Separations among MTQ metabolites were noted in all of the systems.

TABLE I

THIN-LAYER CHROMATOGRAPHY OF MTQ AND MTQOH

Solvent	R_f Observed	
	MTQ	MTQOH
Benzene/n-butanol/methanol (85/10/5)	0.68	0.63
Ethyl acetate/methanol/NH_4OH (85/10/1)	.82	.71
Benzene/acetic acid (9/1)	.38	.17
Ether	.63	.50

IIb RESULTS AND DISCUSSION

Determination of MTQ and MTQOH in Spiked Urine Samples

Urine samples were collected from individuals who had not previously been exposed to the drug. After enzymatic hydrolysis, the samples were spiked with the pure, multilabeled compounds, and extraction and purification steps were performed as described above. The ratios of counts observed at the major peaks of the unlabeled and labeled compounds were only two-to three-fold higher than those obtained with the pure compounds (Figures 1 and 2). Since similar ratios were detected with other urine blanks, it is probable that the sensitivity of the method is primarily limited by the purity of the chromatographic solvents. A major increase in the solvent purity, however, would achieve at best a two- to three-fold decrease in the ratio of counts at the two major masses; consequently, purification of the Spectro-Grade solvents was not attempted.

A calibration curve was obtained by spiking 5 ml samples of hydrolyzed urine with known ratios of labeled and unlabeled MTQ (Figure 3). The count ratio is proportional to the molar ratio to levels as low as 2×10^{-4}. The standard deviation in a point, calculated by least squares analysis, is $\pm 1.6 \times 10^{-5}$. This indicates that MTQ can be determined to a precision of approximately $\pm$ 30 pg/ml.

MTQ and MTQOH Concentrations in Urine After Drug Ingestion

A total of 16 urine samples were collected over a period of 11 days following the ingestion by a volunteer of one 250 mg tablet of Mandrax™. The samples were provided through the courtesy of Col. Douglas J. Beach of the Division of Biochemistry, Walter Reed Army Institute of Research. The levels of MTQ and MTQOH in these samples were analyzed by the methods described.

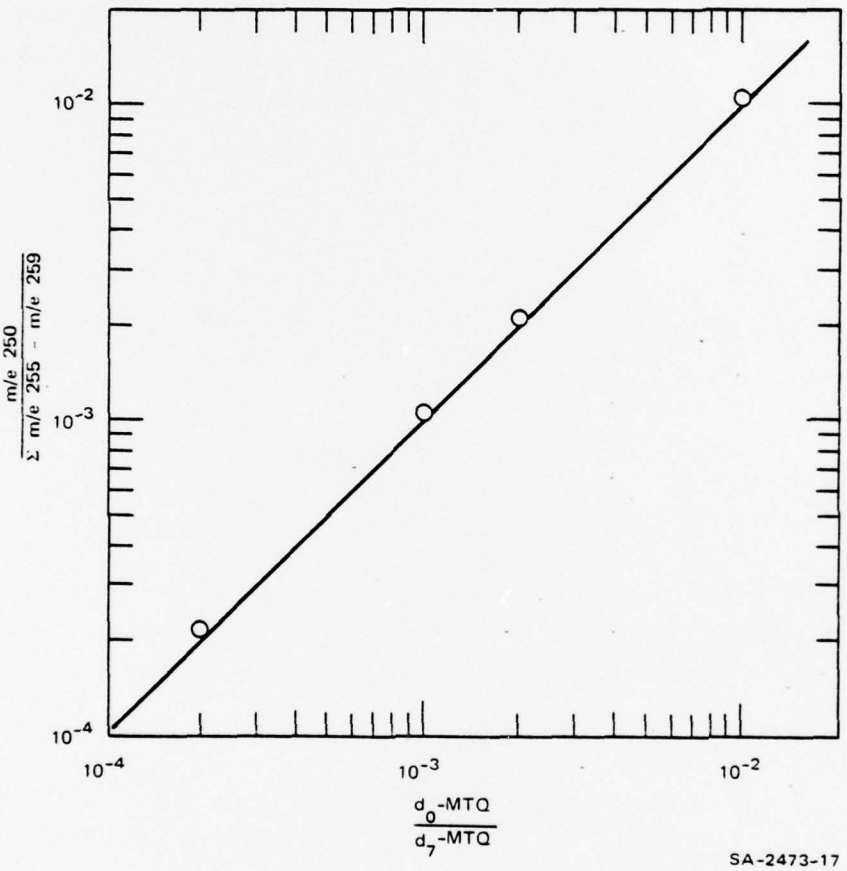


FIGURE 3 CALIBRATION CURVE FOR STANDARD MIXTURES OF d_0- AND d_7-MTQ ADDED TO URINE SAMPLES

The spectrum of total MTQOH (obtained after enzymatic hydrolysis) from the 11th day urine sample is reproduced in Figure 4. It is obvious that a large amount of unlabeled MTQOH is present in this sample, and that the amount of MTQOH is well above the limit of sensitivity of the technique. Analogous results were obtained with MTQ, as shown in Figure 5.

The kinetics of excretion of MTQ, total MTQOH, and unconjugated MTQOH over the 11 day period are illustrated in Figure 6. The half-life corresponding to the slow phase of excretion of the three compounds is approximately 50 hr. The ratio of counts at the unlabeled and labeled mass peaks of MTQ in the eleventh day sample is approximately ten times higher than that observed with control samples. Considering that unchanged MTQ is sometimes undetectable in urine by more conventional analytical procedures,[4] the sensitivity of this analysis makes it particularly valuable for long-term pharmacokinetic investigations.

Sensitivity of MTQ and MTQOH Determination by Isotope Dilution with Multilabeled Standards

The present study shows that the limit of detection of MTQ and MTQOH in urine is approximately 200 pg/ml. This high sensitivity exceeds any of the known methods of MTQ analysis by at least one order of magnitude. Three factors contribute to this sensitivity: (1) rigorous pre-purification of the MTQ and MTQOH by multiple tlc; (2) the use of multilabeled internal standards, in which the level of unlabeled compounds is below 0.01%; and (3) analysis of isotopic ratios by field ionization mass spectrometry, which produces molecular ions of MTQ and MTQOH without measurable fragmentation.

Long-Term Excretion of MTQ and MTQOH

The results of the present study, in which MTQ and MTQOH were determined in urine for 11 days following a single oral dose, complement and extend the earlier investigations of Alvan and collaborators[17] on the concentrations

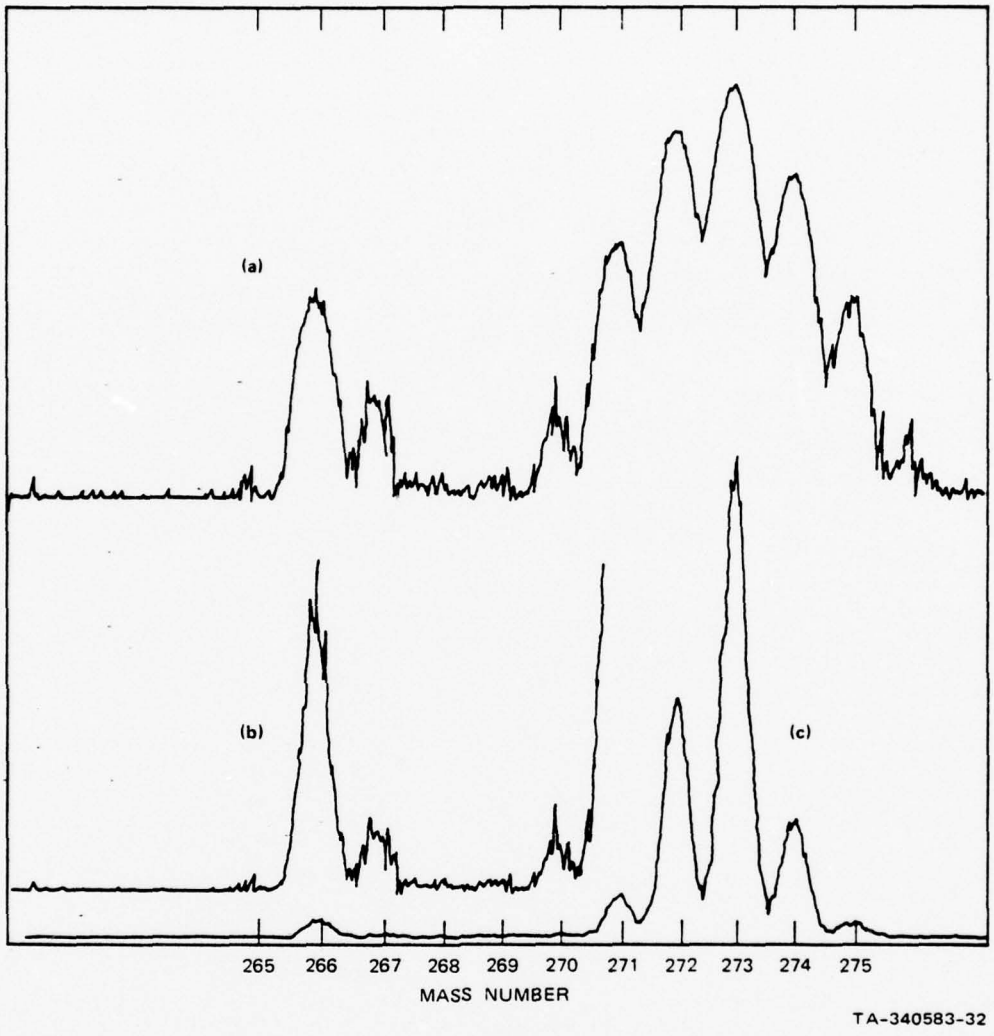


FIGURE 4 FIELD IONIZATION MASS SPECTRA OF MTQOH AND d_7-MTQOH EXTRACTED FROM HYDROLYZED URINE SAMPLE COLLECTED 261.5 HR AFTER DRUG INGESTION: (a) LOGARITHMIC SCALE; (b) LINEAR SCALE, 256 COUNTS/CHANNEL FULL SCALE; (c) LINEAR SCALE, 4000 COUNTS/CHANNEL FULL SCALE

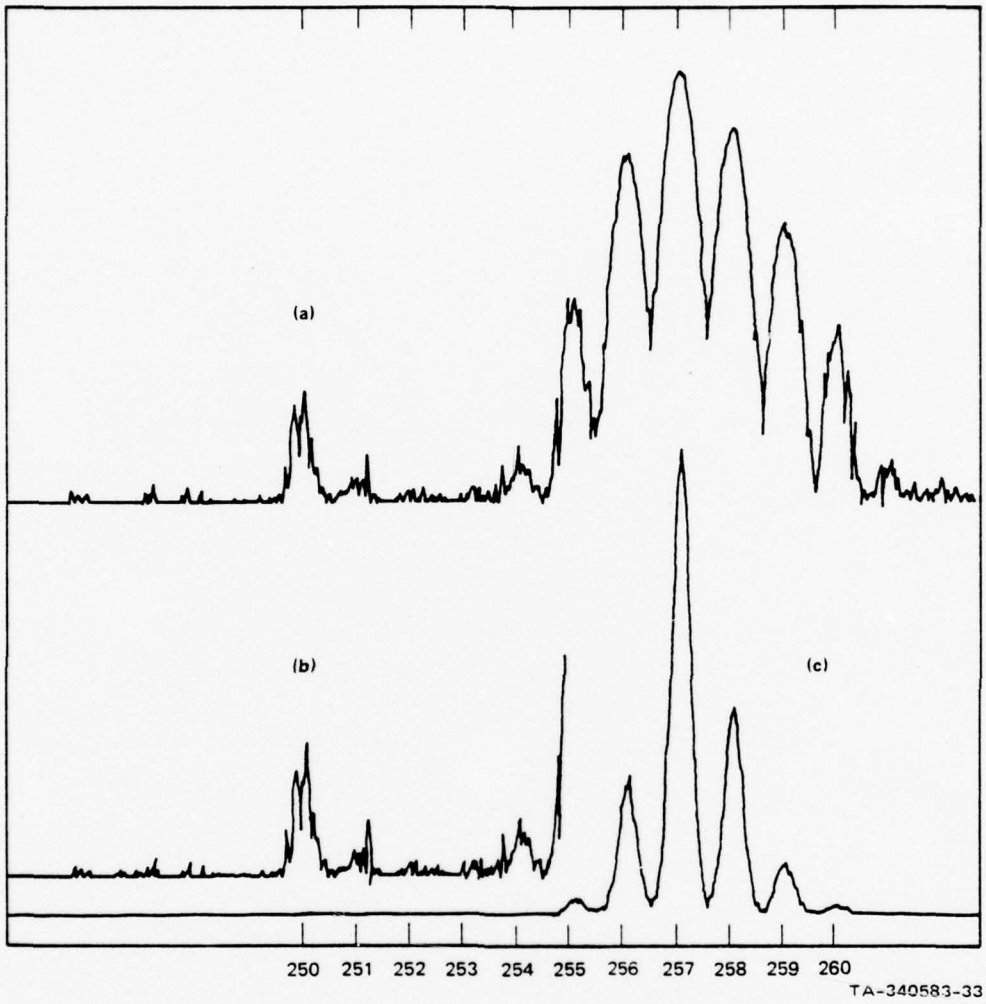


FIGURE 5 FIELD IONIZATION MASS SPECTRA OF MTQ AND d_{10}-MTQ (REDUCED TO d_7 BY EXCHANGE: SEE EXPERIMENTAL) EXTRACTED FROM HYDROLYZED URINE SAMPLE COLLECTED 142 HOURS AFTER DRUG INGESTION: (a) LOGARITHMIC SCALE; (b) LINEAR SCALE, 256 COUNTS/CHANNEL FULL SCALE; (c) LINEAR SCALE, 8000 COUNTS/ CHANNEL FULL SCALE

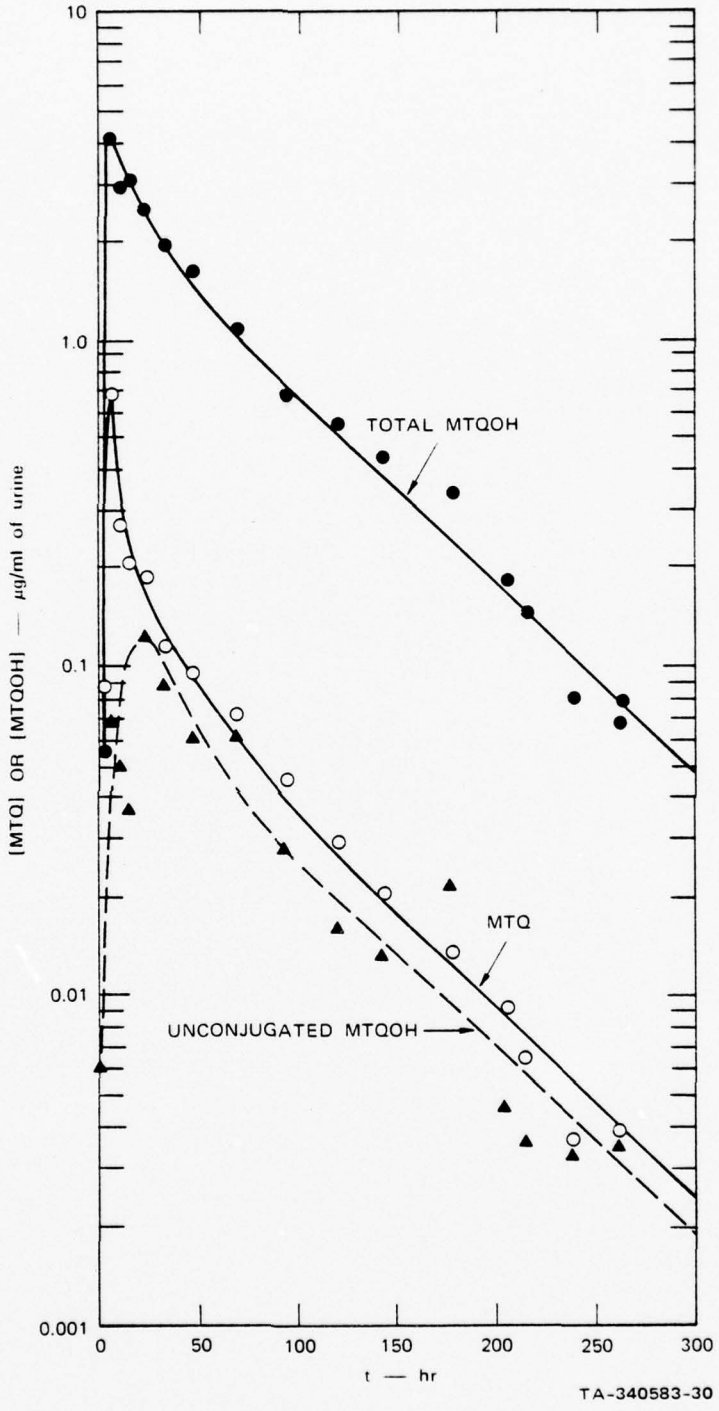


FIGURE 6 ELIMINATION KINETICS OF MTQ AND MTQOH FOLLOWING INGESTION OF A SINGLE 250 mg TABLET

of MTQ in plasma over a 5 day period. Our results support the conclusions of the Karolinska group that MTQ persists for extended periods in the human body. Indeed, the half-life that we measured for the slow phase of excretion is even longer than reported by the Swedish workers. This could reflect individual variations in excretion rates, or it may signify that a slower phase of excretion develops after 4 or 5 days. The latter hypothesis is supported by Figure 6, which indicates that the slow phase of excretion does not become apparent until at least 100 hours after drug ingestion. These results emphasize the desirability of long-term pharmacokinetic studies, lasting over several weeks, to fully define the spectrum of rate constants involved.

It is also of interest that after approximately two days, the ratio of MTQ/MTQOH in urine remains approximately constant, with the total MTQOH level being always about 20 times higher than that of MTQ. This indicates a slow release of MTQ from an isolated compartment, probably an adipose phase, followed by rapid metabolism and excretion. It is most unlikely that MTQ and its oxidized metabolite are being released from storage pools at identical rates. Moreover, the much higher level of glucuronide than free MTQOH argues against any storage of the hydroxylated derivative. In all probability, therefore, the metabolite of MTQ does not possess the pharmacological activity of the parent drug, the activities of the two compounds presumably being a function of their lipid solubilities.

The concentrations of MTQ and MTQOH in the urine samples have not been corrected for changes in clearance rate, i.e., creatinine content. These changes might account for the observed fluctuations in MTQ and MTQOH. It may be noted that the fluctuations for all three curves occur in the same direction.

III DEVELOPMENT OF AN IMPROVED METHOD OF EXTRACTION AND PURIFICATION OF MTQ AND MTQOH

The methodology given in Section II which was developed for a high-sensitivity isotope dilution analyses involved a urinary extraction by passage through XAD-2 resin, elution from the resin using ethyl acetate/chloroform (3/2),[30] and purification of the eluant by four chromatographies on silica gel plates. Although this methodology yielded samples of very high purity, it was tedious and time-consuming and would be unsuitable for large numbers of samples.

It seemed probable that a reduction in the number of chromatographies could be achieved by a more selective elution from the XAD-2 resin. Accordingly, a series of low polarity solvents were tested for their ability to elute both MTQ and MTQOH from an XAD-2 resin. It was expected that in all cases the eluants would contain compounds having the same mass number as unlabeled MTQ and MTQOH, but that the concentration of these compounds would be low enough to permit their removal by a single two-dimensional tlc step.

The solvents tested are summarized in Table II. It is seen that MTQ is eluted by all solvents tested, including hexane, but that the elution of MTQOH requires a certain minimal solvent polarity. The polarity required, however, is significantly below that previously used for elution of MTQ and MTQOH from XAD-2 resin.

Following selection of an appropriate eluting solvent (either hexane/ether, 75/25, or hexane/isopropanol 95/5; Table II), a series of urine blanks were spiked with carrier levels of multilabeled MTQ* and MTQOH*. The urines were hydrolyzed with β-glucuronidase/aryl sulfatase as described in Section II, extracted with XAD-2 resin, and eluted with the desired solvent system.

TABLE II

ELUTION OF MTQ AND MTQOH FROM XAD-2
RESIN WITH DIFFERENT ORGANIC SOLVENTS

Solvent	Elution Capability[a]	
	MTQ	MTQOH
Hexane	+	-
Hexane/ether (95/5)	+	-
" (85/15)	+	+-
" (75/25)	+	+
Hexane/isopropanol (95/5)	+	+

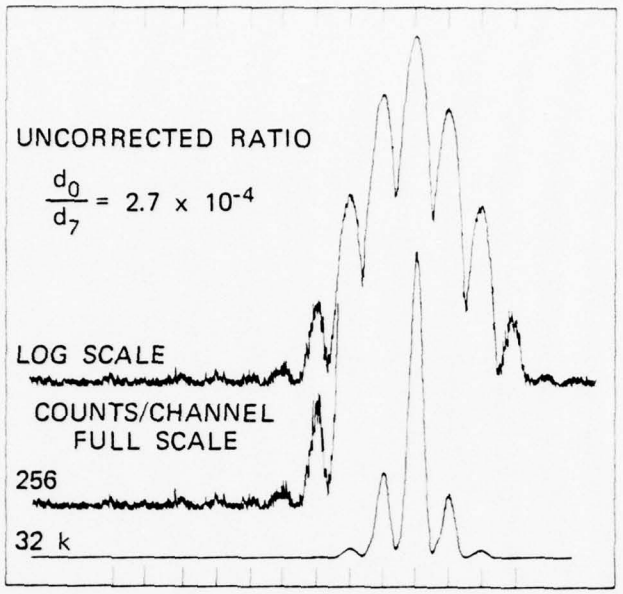
[a]The tests were performed using small columns (Bio-Rad 731-1110) of XAD-2. In each case, the compounds dissolved in water were extracted onto the resin, then 20 ml of the appropriate solvent were passed through the column. After concentrating the eluant to dryness, analysis was performed by tlc (benzene/isopropanol, 95/5)

Purification of the eluants was carried out by two-dimensional tlc (benzene/isopropanol, either 85/15, 90/10, or 95/5, followed by ether). Of these tlc solvents, the benzene/isopropanol (95/5) mixture eventually yielded the cleanest mass spectra. This was somewhat surprising, since the migration of the spots, and therefore, their separation, was least in this system.

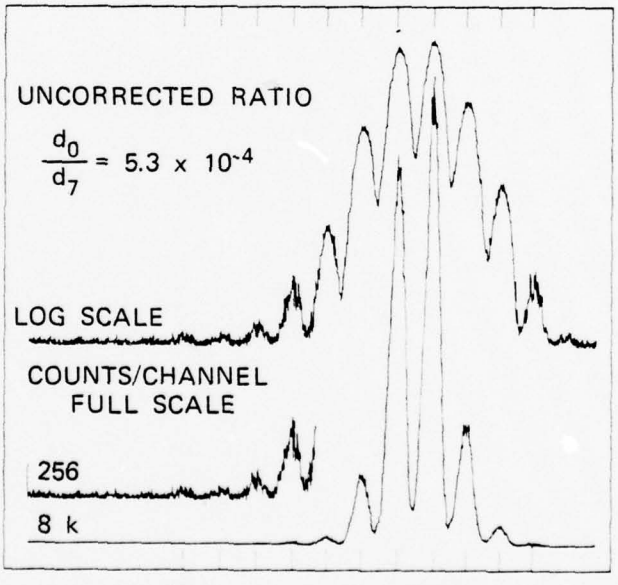
More surprising, however, was the apparent disparity between the cleanliness of the tlc plates and the cleanliness of the product mass spectra. It was observed that the hexane/ether (75/25) eluants from the XAD-2 resin invariably yielded fewer spots on the tlc plates than did the hexane/isopropanol (95/5) eluants. It was expected, therefore, that the product purity would be higher in the case of the hexane/ether extracts.

Examination of the mass spectra of the products revealed, however, that the highest purity was reached following elution from the resin with hexane/isopropanol (95/5). Spectra obtained with this system of elution followed by two-dimensional tlc (benzene/isopropanol, 95/5; ether) are shown in Figure 7. The uncorrected ratios, illustrated in the figure, are almost identical to those obtained after elution with ethyl acetate/chloroform (3/2) and four-dimensional tlc.

To further substantiate the high purities attainable by a technique of selective elution from XAD-2 resin and two-dimensional tlc, a series of urine samples were spiked with known ratios of MTQ/MTQ* and MTQOH/MTQOH*. After enzymatic hydrolysis, extraction, and chromatography, the apparent ratios were calculated from the multiscanned, field ionization mass spectra. The dilution series are shown in Figure 8, which compares the mass spectrometric count ratio with the molar ratio for the two compounds. The calculated least squares line, shown in the figure, demonstrates the expected proportionality between these two parameters, and proves that the results are accurate in the nanogram range.



(a) METHAQUALONE-d_7



(b) 6-HYDROXYMETHAQUALONE-d_7

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FIGURE 7 FIELD IONIZATION MASS SPECTRA OF HEPTALABELED MTQ (A) AND MTQOH (B) AFTER EXTRACTION FROM HYDROLYZED URINE AND 2-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

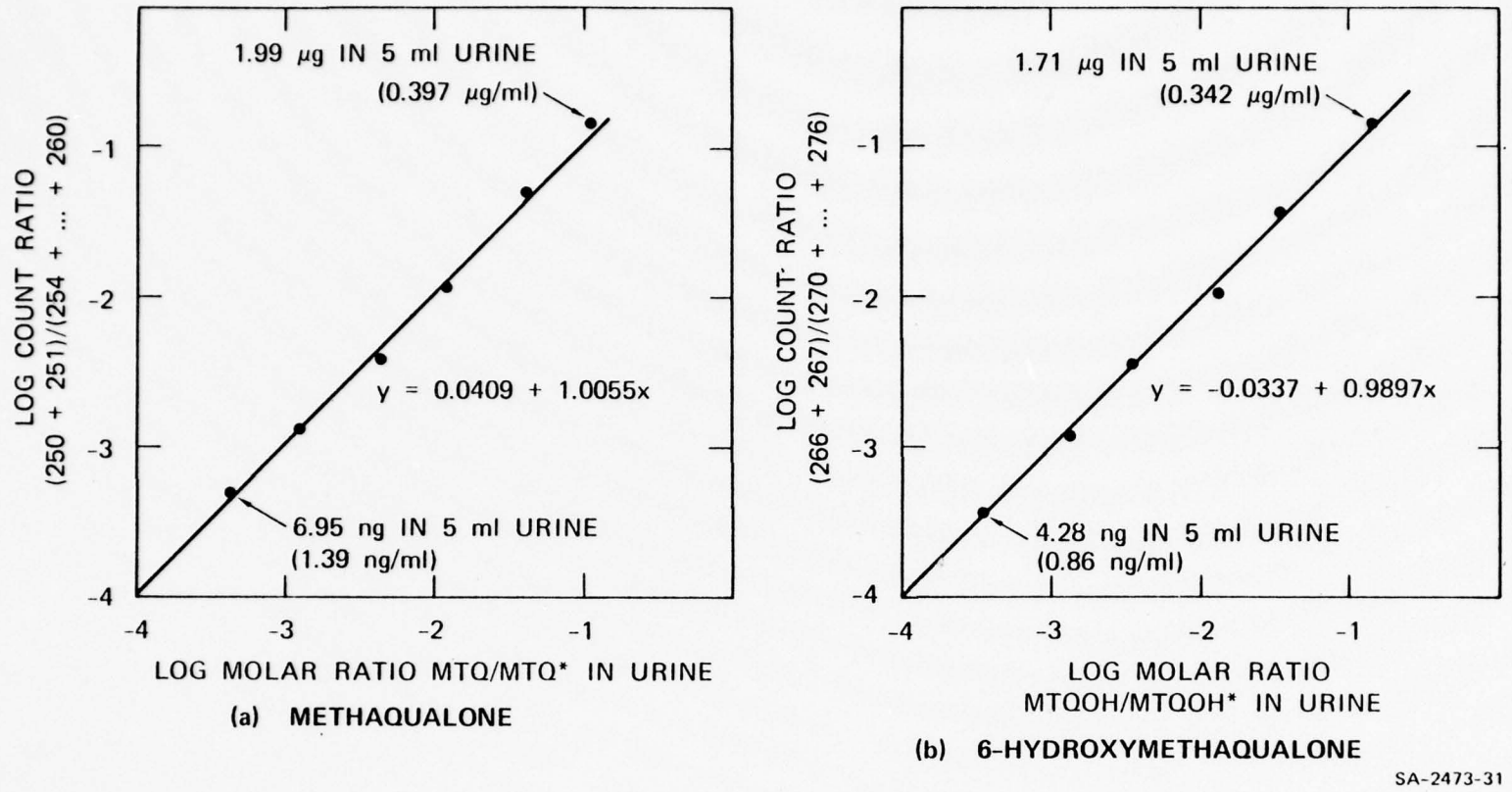


FIGURE 8 STANDARD DILUTION SERIES FOR MTQ (A) AND MTQOH (B) EXTRACTED FROM HYDROLYZED URINE AND PURIFIED BY 2-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

Application of the Improved Extraction Procedure to the Analysis of Urine Samples

A series of 58 urine samples were collected from an individual following ingestion of one tablet of MTQ. The sample numbers were assigned randomly and did not correspond to the order in which the urine samples were obtained. Each sample was approximately 10 ml in volume.

The methodology used for treatment of the urines was identical to that given in Section II under the heading "Extraction of MTQ and MTQOH from Urine," up to the point where the urine samples have been extracted onto the XAD-2 resin. Elution of the samples from the columns was, however, performed using hexane/isopropanol (95/5), rather than the former ethyl acetate/chloroform (3/2).

MTQ and MTQOH were isolated from the concentrated eluants by bidimensional chromatographies on commercial silica gel plates that contained a fluorescent indicator (Analtech 2011). The plates were initially washed with methanol, then were activated by heating at 110°C for approximately one hour. The solvents used and the observed R_f values are given in Table III.

TABLE III

THIN-LAYER CHROMATOGRAPHY OF MTQ AND MTQOH

Solvent	R_f Observed	
	MTQ	MTQOH
Benzene/isopropanol (95/5)	0.32	0.19
Ether	.63	.50

It should be emphasized that in the solvent systems tested, separation of the major hydroxylated MTQ derivatives appears to be complete. In the hydrolyzed samples that were collected only a few hours after drug

ingestion, at least four other major spots were visible, in addition to the 6-hydroxy isomer, that were not detectable in later urine samples. In all probability, these spots are hydroxylated MTQ derivatives: Sleeman *et al.*[10] have identified the four major hydroxylated MTQ derivatives detectable by TLC, one of which is the 6-hydroxy isomer. Other investigators have also reported thin-layer separations of various products of MTQ oxidation.[3]

The similarities between our observations and those reported by others indicate that the solvent systems used separate at least the major hydroxylated derivatives of MTQ from one another. Possible overlap of the 6-hydroxy isomer with a minor isomer of MTQOH present at much lower levels is not ruled out, however. Nevertheless, if this occurs, the level of interference should be low, and should not materially alter the results.

IV RESULTS

Determination of MTQ, Total MTQOH, and Unconjugated MTQOH Concentrations in Urine Samples

As indicated in Section III, the 58 urine samples were identified by number only, the numbers having been assigned randomly to the series by the Biochemistry Laboratory of the Walter Reed Hospital before supplying them to us. Consequently, we knew neither the times at which the samples were obtained nor the order of their collection.

Of the 58 urine samples, 55 were test samples collected during a one-month period following drug ingestion. Three of the samples were controls containing aliquots from successive 24-hour urine collections obtained during the 3-day period immediately before drug ingestion. These controls were distributed randomly among the 55 test samples; their identities as well as the identities of the test samples, remained unknown until the end of the experiment.

The presence of three controls provided a check on the "carryover" or "memory" involved in the total analytical procedure. To minimize memory effects, the mass spectrometer was baked out between runs, and a sample of the pure, multilabeled compound being used was analyzed by multiscanning, field ionization mass spectrometry between each urine sample. The spectrum of the pure compound was repetitively scanned until the ratio of counts at the unlabeled and labeled mass peaks had fallen to approximately 1×10^{-4}. For a 10 μg sample of labeled compound, this implies that the allowed residual level of unlabeled MTQ or MTQOH was approximately 1 ng. It will be seen that this level was approximately the amount "found" in the three control urine samples.

A summary of the concentrations of MTQ and total MTQOH is provided in Tables IV and V. The third column of each table shows the actual measured concentrations of compound, while the fourth column shows the corrected concentrations after subtraction of the average residual level

TABLE IV. CONCENTRATIONS OF MTQ IN URINE

Order of Collection	Sample Number	[MTQ] Measured (ng/ml)	[MTQ] Corrected (ng/ml)
	17	2.6	
	58	0.1	
	50	0.4	
	(CONTROLS)	Ave. 1.0 ± 1.1 (s.d.)	
1	15	2,042	2,041
2	47	973.3	972.3
3	13	895.6	894.6
4	57	348.0	347.0
5	52	157.6	156.6
6	56	345.7	344.7
7	46	52.3	51.3
8	3	183.0	182.0
9	31	159.2	158.2
10	22	163.7	162.7
11	6	177.0	176.0
12	20	175.6	174.6
13	44	22.1	21.1
14	24	33.7	32.7
15	36	53.3	52.3
16	33	32.4	31.4
17	2	100.0	99.0
18	29	346.7	345.7
19	54	96.6	95.6
20	12	73.2	72.2
21	38	38.2	37.2

TABLE IV. CONCENTRATIONS OF MTQ IN URINE (continued)

Order of Collection	Sample Number	[MTQ] Measured (ng/ml)	[MTQ] Corrected (ng/ml)
22	21	102.7	101.7
23	51	67.1	66.1
24	25	78.9	77.9
25	23	75.4	74.4
26	8	48.2	47.2
27	4	62.0	61.0
28	19	62.9	61.9
29	37	30.3	29.3
30	55	42.1	41.1
31	35	53.0	52.0
32	43	44.3	43.3
33	42	31.5	30.5
34	27	18.8	17.8
35	10	18.6	17.6
36	48	19.1	18.1
37	32	7.4	6.4
38	16	10.6	9.6
39	40	4.5	3.5
40	5	5.5	4.5
41	34	4.4	3.4
42	1	3.1	2.1
43	26	3.4	2.4
44	14	3.1	2.1
45	41	1.3	0.3
46	53	1.5	0.5
47	7	1.9	0.8
48	45	1.8	0.7

TABLE IV. CONCENTRATIONS OF MTQ IN URINE (continued)

Order of Collection	Sample Number	[MTQ] Measured (ng/ml)	[MTQ] Corrected (ng/ml)
49	11	1.9	0.8
50	39	4.9	3.9
51	18	7.7	6.7
52	28	1.1	0.1
53	30	1.3	0.3
54	9	2.0	1.0
55	49	0.2	0.

TABLE V. CONCENTRATIONS OF TOTAL MTQOH
(AFTER ENZYMATIC HYDROLYSIS) IN URINE

Order of Collection	Sample Number	[MTQOH] Measured (ng/ml)	[MTQOH] Corrected (ng/ml)
	17	3.3	
	58	1.2	
	50	1.7	
	(CONTROLS)	Ave. 2.1 ± 0.9 (s.d.)	
1	15	10,895	10,893
2	47	1,945	1,943
3	13	824.7	822.6
4	57	5,585	5,583
5	52	1,044	1,042
6	56	12,307	12,305
7	46	3,055	3,053
8	3	4,654	4,652
9	31	1,588	1,586
10	22	2,015	2,013
11	6	2,019	2,017
12	20	3,709	3,707
13	44	[49,649]	[1,503][a]
14	24	106.4	104.3
15	36	187.6	185.5
16	33	145.9	143.8
17	2	-	[801][b]
18	29	771.0	768.9
19	54	1,015	1,013

TABLE V. CONCENTRATIONS OF TOTAL MTQOH
(AFTER ENZYMATIC HYDROLYSIS) IN URINE (Continued)

Order of Collection	Sample Number	[MTQOH] Measured (ng/ml)	[MTQOH] Corrected (ng/ml)
20	12	968	965.9
21	38	622.2	620.1
22	21	858.9	856.8
23	51	523.6	521.5
24	25	439.4	437.3
25	23	1,298	1,296
26	8	497.0	494.9
27	4	1,248	1,246
28	19	427.0	424.9
29	37	155.2	153.1
30	55	544.4	542.3
31	35	-	[396][b]
32	43	311.1	309.0
33	42	287.9	285.8
34	27	192.5	190.4
35	10	250.0	247.9
36	48	175.7	173.6
37	32	118.6	116.5
38	16	108.5	106.4
39	40	78.6	76.5
40	5	93.5	91.4
41	34	41.7	39.6
42	1	83.9	81.8
43	26	30.3	28.2

TABLE V. CONCENTRATIONS OF TOTAL MTQOH
(AFTER ENZYMATIC HYDROLYSIS) *IN URINE* (*Continued*)

Order of Collection	Sample Number	[MTQOH] Measured (ng/ml)	[MTQOH] Corrected (ng/ml)
44	14	15.2	13.1
45	41	24.4	22.3
46	53	12.9	10.8
47	7	11.9	9.8
48	45	12.4	10.3
49	11	11.8	9.7
50	39	7.2	5.1
51	18	5.1	3.0
52	28	6.1	4.0
53	30	4.4	2.3
54	9	3.1	1.0
55	49	2.2	0.1

[a]Measured value was rejected owing to a very low level of multilabeled carrier in the extract. Sample 13 apparently received much less than the normal carrier dose, or may have received none at all. The corrected [MTQOH] value was estimated from a plot of the average excretion rate *vs.* time (Figure 4). [b]Sample lost. Corrected value estimated from a plot of the average excretion rate *vs.* time (Figure 4).

"found" in three control urines.

After completion of the series, the code was revealed to us. Forty of the samples were then analyzed for their concentrations of unconjugated MTQOH. In this case, since the code was known, the samples were analyzed in a decreasing time sequence, analyzing the earliest sample last so as to minimize memory effects. Generally increasing levels of unconjugated MTQOH are expected for this sequence (Section II), which permitted a more rapid sample throughput. The first sample measured was a control, and indicated undetectable MTQOH. Therefore, no corrections to the measured MTQOH concentrations were made. The results are summarized in Table VI.

The volumes of urine, the collection intervals, and the creatinine levels, are summarized in Table VII. These data were provided by the sponsors, and are necessary for an eventual pharmacokinetic analysis of the results.

TABLE VI. CONCENTRATIONS OF UNCONJUGATED MTQOH IN URINE

Order of Collection	Sample Number	Unconjugated [MTQOH] (ng/ml)
	50 (CONTROL)	0.00
1	15	381.4
2	47	1,870
3	13	803.6
4	57	101.7
5	52	692.6
6	56	1,349
7	46	92.2
8	3	42.2
9	31	56.6
10	22	29.5
11	6	105.0
12	20	55.2
13	44	9.35
14	24	36.9
15	36	15.3
16	33	11.7
17	2	57.9
18	29	16.4
19	54	22.7
20	12	16.4
21	38	17.5
22	21	95.0
23	51	21.0

TABLE VI. CONCENTRATIONS OF UNCONJUGATED MTQOH IN URINE (continued)

Order of Collection	Sample Number	Unconjugated [MTQOH] (ng/ml)
24	25	10.2
25	23	25.0
26	8	19.?
27	4	20.8
28	19	2.79
29	37	1.46
30	55	20.5
31	35	20.4
32	43	26.6
33	42	17.3
34	27	7.77
35	10	8.41
36	48	7.63
37	32	2.87
38	16	6.64
39	40	2.31

TABLE VII. URINE VOLUMES, COLLECTION INTERVALS, AND CREATININE LEVELS FOR SAMPLES 1-58

Sample Number	Collection Interval (hr)	Total Volume (ml)	Creatinine (mg %)
17	-72 - (-48)	1440	124
58	-48 - (-24)	935	188
50	-24 - 0	1050	164
(CONTROLS)			
15	0 - 2	83	96
47	2 - 3	272	20
13	3 - 4	375	12
57	4 - 6	265	54
52	6 - 7.75	375	30
56	7.75 - 10	150	72
46	10 - 14	185	138
3	14 - 22	375	108
31	22 - 26	245	120
22	26 - 29	155	80
6	29 - 34	135	210
20	34 - 38	160	266
44	38 - 46	255	140
24	46 - 50	265	120
36	50.5 - 52.5	274	38
33	52.5 - 53.5	265	32
2	53.5 - 56	230	84
29	56 - 61.5	245	120

TABLE VII. URINE VOLUMES, COLLECTION INTERVALS, AND CREATININE LEVELS FOR SAMPLES 1-58 (Continued)

Sample Number	Collection Interval (hr)	Total Volume (ml)	Creatinine (mg %)
54	61.5 - 65	125	170
12	65 - 73.5	515	100
38	73.5 - 78.5	215	148
21	78.5 - 80.5	80	118
51	80.5 - 86	320	160
25	86 - 89.33	205	92
23	89.33 - 95.83	295	156
8	95.83 - 100	110	116
4	100 - 105.5	180	116
19	105.5 - 108	120	170
37	108 - 110.75	170	150
55	110.75 - 120	380	136
35	120 - 144	1045	144
43	144 - 168	995	180
42	168 - 192	1050	96
27	192 - 216	1260	132
10	216 - 240	1050	164
48	240 - 264	1275	122
32	264 - 288	1150	144
16	288 - 312	1420	132
40	312 - 336	1215	134
5	336 - 360	970	156
34	360 - 384	1270	128

TABLE VII. URINE VOLUMES, COLLECTION INTERVALS, AND CREATININE LEVELS FOR SAMPLES 1-58 (Continued)

Sample Number	Collection Interval (hr)	Total Volume (ml)	Creatinine (mg %)
1	384 - 408	1270	128
26	408 - 432	1490	128
14	432 - 456	1360	124
41	456 - 480	915	152
53	480 - 504	1200	118
7	504 - 528	965	200
45	528 - 552	965	200
11	552 - 576	935	232
39	576 - 600	1200	168
18	600 - 624	900	196
28	624 - 648	800	214
30	648 - 672	935	164
9	672 - 696	975	198
49	696 - 720	770	206

V DISCUSSION

Figure 9 illustrates the average urinary concentrations of MTQ, unconjugated MTQOH, and total MTQOH plotted as a function of time at the midpoints of the collection intervals. It is obvious that the scatter is very large, and qualitatively at least, there does not appear to be any correlation between the variations shown by one compound and those exhibited by another. This suggests that variations in kidney function are probably not the major cause of scatter. It does not, therefore, seem particularly useful or valid to normalize the concentrations to those found for creatinine.

An alternative representation of the data, which is more conventional in representing urinary concentrations of drugs,[31] is to plot the average excretion rates vs. the time at the midpoint of the collection intervals. Such a plot is shown in Figure 10. Again, the scatter is seen to be quite large, and is apparently not correlated from compound to compound.

In both Figures 9 and 10, the half-lives corresponding to the terminal excretion phases are given, and the agreement is adequate. It should be stressed that the lines were drawn visually, and were not calculated by least-squares procedures. Therefore, the estimated terminal half-lives are merely approximate, and could be in error by as much as 10%. Despite this uncertainty, however, it is obvious that the loss of MTQ from the body is in this instance markedly slower than has been reported by others as well as by ourselves (Section II).[17,20,22] The difference between our previous estimate of approximately 50 hours for the terminal half-life, and the present estimates of approximately 70 hours appear to be within a reasonable range of biological variability. On the other hand, these estimated half-lives are much higher than those determined by previous workers, based on more limited pharmacokinetic data.[17,20,22]

The present experiments are the first instance in which the excretion of MTQ was followed for as long as 10 half-lives. The signifncantly shorter half-lives obtained by others are very probably due to the shorter time periods

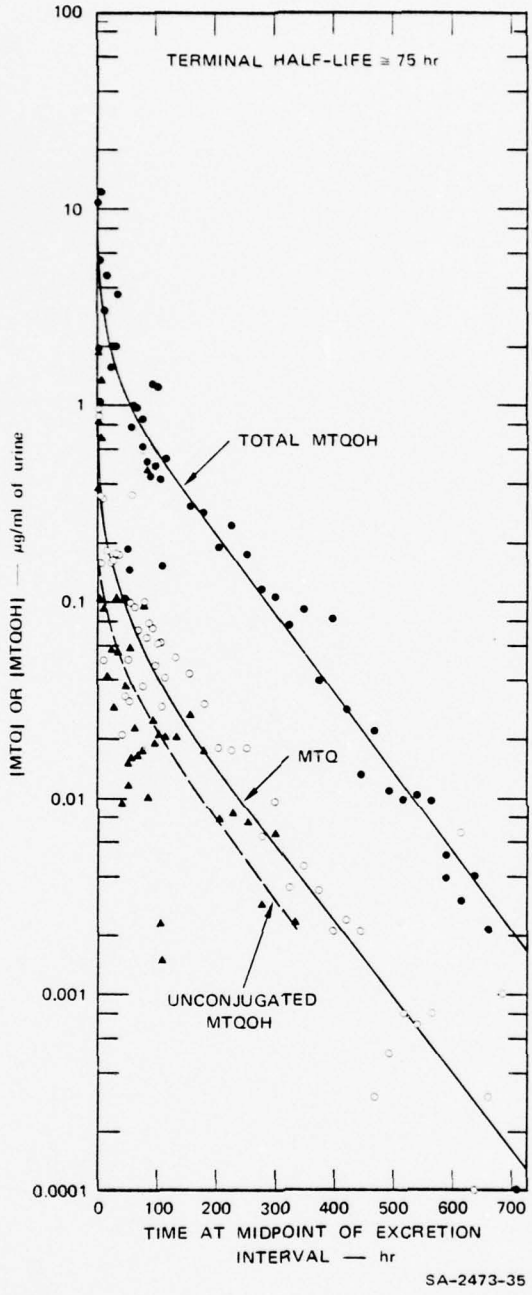


FIGURE 9 CONCENTRATIONS OF METHAQUALONE, TOTAL 6-HYDROXYMETHAQUALONE, AND UNCONJUGATED 6-HYDROXY-METHAQUALONE IN URINE VERSUS TIME AT MIDPOINT OF EXCRETION INTERVAL

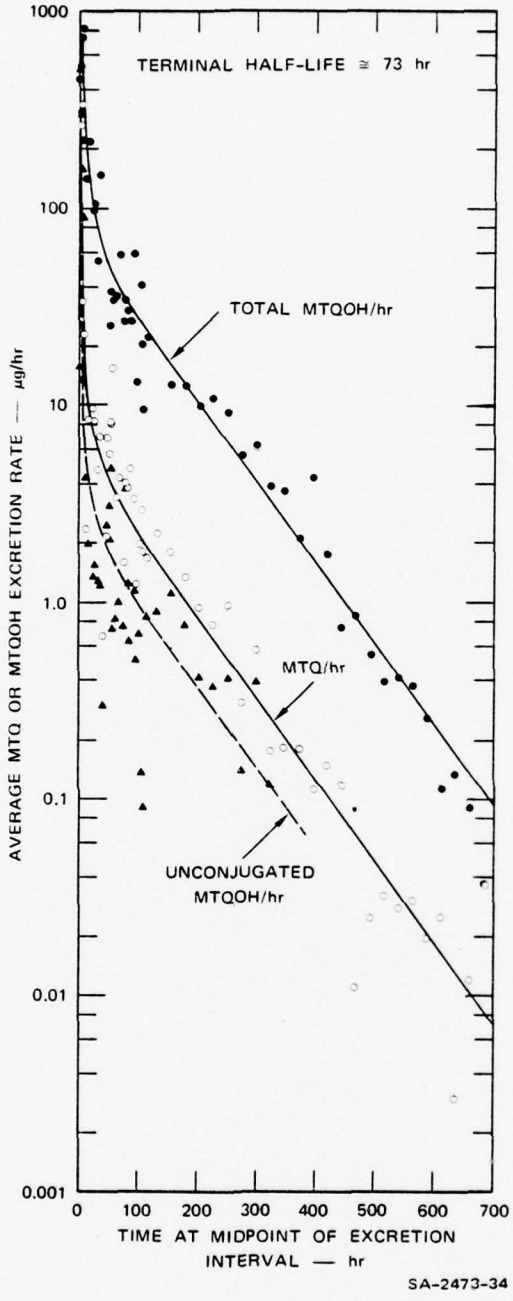
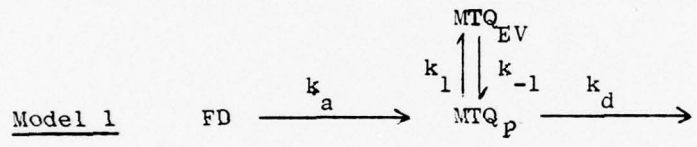


FIGURE 10 AVERAGE EXCRETION RATES OF METHAQUALONE, TOTAL 6-HYDROXYMETHAQUALONE, AND UNCONJUGATED 6-HYDROXY-METHAQUALONE AFTER INGESTION OF A SINGLE 300 mg TABLET

over which pharmacokinetic data were accumulated; kinetic measurements were were rarely made over more than three half-lives, and in one case, less than two half-lives "sufficed" for the estimate of the terminal rate constant.[22] It is well recognized that a slowly varying exponential decay curve may appear linear if relatively few data points are obtained, and if the experimental scatter is large. This seems to be the most probable explanation for the discrepancy between our half-life estimates and those reported by others.

Preliminary Pharmacokinetic Interpretation

Two groups of investigators[17,22] have utilized a conventional two-compartment open model to analyze the pharmacokinetics of MTQ over a more limited time range than was studied here. This model is summarized schematically below:



where F is the fraction of the dose, D, ultimately absorbed, hence FD is the amount absorbed, MTQ_P signifies the drug in the central compartment (plasma), and MTQ_{EV} corresponds to the drug in an extravascular compartment, the nature of which is not specified. The rate constants are all first-order constants signifying, respectively, absorption from the gut (k_a), transfer or distribution from plasma to the extravascular space (k_1), reverse transfer (k_{-1}), and elimination by all processes including metabolism and excretion (k_d). Such a model is characterized by three exponential terms, e.g.,

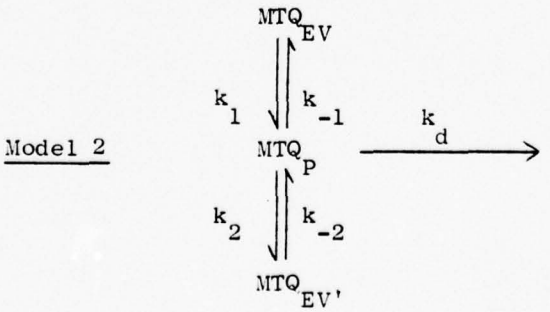
$$[MTQ_P] = A_1 e^{-\alpha t} + A_2 e^{-\beta t} + A_3 e^{-k_a t} \qquad (1)$$

where α and β are composite functions of the rate constants for disposition and elimination.[32] In general, α is identified with the fast component

of disposition and β with the terminal exponential decline.

Previous investigators have obtained values for these constants from plasma concentration curves of MTQ in normal subjects, and the results obtained by different laboratories are reasonably consistent. The results show that the absorption phase is essentially completed within about 3 hr ($k_a \cong 0.8\ hr^{-1}$),[22] and that the rapid distribution phase is over for most subjects within approximately 10 hours ($\alpha_{min} \cong 0.34\ hr^{-1}$).[17] Consequently, according to this model, all data collected after about 10 hours should be characterized by a single exponential decay constant, β. Inspection of Figure 9 shows clearly, however, that this is not the case. The terminal phase of excretion is not attained until at least 100 hours after drug ingestion. This was observed previously by us in the initial study which covered an 11-day excretion period (Section II). The present, more extensive investigation leads to an identical conclusion. It follows that the conventional two-compartment open model is inadequate to describe the pharmacokinetics of MTQ.

The logically next higher level of complexity would involve a three-compartment open model, of which a number of possibilities may be considered. Several such models are suggested in standard pharmacokinetic texts,[33,34] and others can be devised. Two possible models are discussed below. In both cases, for simplicity, the rapid absorption phase has been neglected, and only the distribution phases are considered. The models can be applied, therefore, only to data collected after the initial 3 hr.



In this model, MTQ_{EV} and $MTQ_{EV'}$, signify methaqualone distributed in two different extravascular compartments, the first-order rate constants, k_1, k_{-1}, k_2 and k_{-2} characterize the transfer of MTQ between plasma and these compartments, and k_d has the same meaning previously assigned. This model is considered in detail by Rescigno and Segre.[35] It has been applied previously to the distribution of radioiodine among three compartments (blood, thyroid, and "tissues")[36] and to the distribution of ^{131}I-labeled albumin between plasma and two different extravascular spaces.[37]

The model predicts that in the postabsorptive phase, the MTQ concentration will be characterized by three exponentials, e.g.,

$$[MTQ_P] = B_1 e^{-b_1 t} + B_2 e^{-b_2 t} + B_3 e^{-b_3 t} \qquad (2)$$

where the b_i's and B_i's are known functions of the rate constants and initial concentrations.[35] (It will be recalled that the two-compartment model was characterized by only two exponentials in the postabsorptive phase.) It is obvious that at least three exponential terms are needed to fit the data obtained subsequent to drug absorption (i.e., $t > 3$ hr).

From a physiological standpoint, a three-compartment model is undoubtedly more realistic than a two-compartment model. It is quite likely that MTQ could be distributed in significant amounts in more than one compartment, and that different compartments would absorb and release the drug at different rates. These considerations lend logical support to the type of scheme shown in Model 2.

There are, however, some logical discrepancies in this model as well. One difficulty is that the model implies that drug elimination occurs only from the central compartment. ("Elimination" in a pharmacokinetic sense includes both metabolism and excretion; the former is the major route of MTQ elimination.) It is known, however, that the relevant drug metabolizing enzymes are membrane-bound, being located largely in the

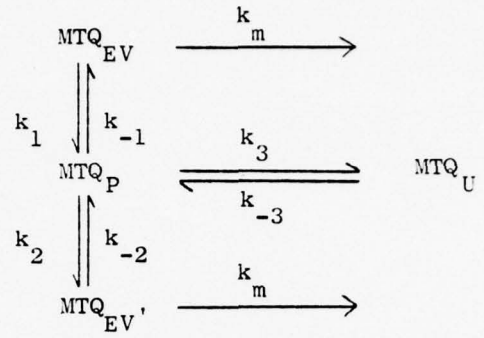
endoplasmic reticulum fraction of the liver. It is possible, of course, to modify the model such that drug metabolism is specifically stated to occur in one or both of the extravascular compartments, but the number of rate constants would then be increased, and a unique determination of all rate constants would no longer be possible based on plasma (or urine) data alone.

Even this modification, however, would not eliminate all of the logical objections to Model 2. A further objection can be raised on the grounds that excretion is depicted as being irreversible. It is well-known, however, that for highly lipophilic compounds such as MTQ, excretion is a very inefficient process, and that extensive reabsorption occurs through the membranes surrounding the distal tubules of the kidney back eventually into plasma.[38,39] Indeed, it has been suggested that the very long half-lives exhibited by some lipophilic drugs, as well as their correspondingly high levels of metabolic transformation, are largely caused by inefficient renal excretion.[38,39]

An additional factor working against rapid excretion of MTQ is the extent of plasma protein binding. Lipophilic drugs such as MTQ are strongly bound to plasma proteins, such as serum albumin. It is only the free (unbound) drug, however, that can be filtered by the kidney. Estimates of the extent of plasma protein binding of MTQ have been made,[40] and indicate that in the therapeutic range the percentage of bound drug is approximately 90%.

These considerations suggest that another model, which takes passive tubular reabsorption in the kidney specifically into account, may provide a more consistent means of analyzing the pharmacokinetic data. Model 2 could, of course, be modified appropriately, viz.:

Model 2'

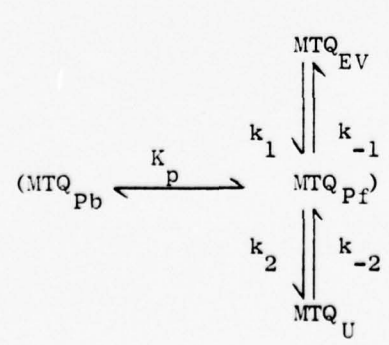


where, now, metabolism (signified by k_m) is shown to occur only in extravascular compartments, and excretion of MTQ from plasma (k_3) is shown to be a reversible process. The presence of MTQ in urine is denoted by MTQ_U.

This modification of Model 2 seems to satisfy most of the logical objections that have been raised. As shown, however, it is now a four-compartment open model, and will be characterized by four exponentials in the postabsorptive phase. With increasing numbers of exponentials, the scatter in the data can become of paramount importance for deciding what constitutes an acceptable fit. Unfortunately, it is unlikely that the scatter in this case would permit a three-compartment model to be ruled out.

A simplification of the model, which retains the major logical modifications introduced above, but requires only three exponential terms, can be achieved simply by dropping one of the postulated extravascular compartments. By doing so, we obtain Model 3.

Model 3



$$K_P = \frac{[MTQ_{Pf}]}{[MTQ_{Pb}]}$$

Model 3 is a three-compartment open model in which one of the compartments is the urine. Without further information, the other compartment may be regarded simply as "tissues". This model specifically incorporates the objections raised against the preceding model: (1) metabolism occurs in the tissues rather than in the plasma (k_m); (2) passive tubular reabsorption occurs (k_{-2}); and (3) the relevant plasma concentration governing the rate of distribution of MTQ to the two extravascular compartments is the free drug level (MTQ_{Pf}). Free drug is, of course, in rapid equilibrium with the bound fraction (MTQ_{Pb}), the equilibrium constant being denoted here by $K_P = [MTQ_{Pf}]/[MTQ_{Pb}]$. It will be useful (see below) to recognize this equilibrium explicitly for the case of plasma.

Pharmacokinetic Analysis of Model 3

To decide whether the pharmacokinetic data can be rationalized in terms of Model 3, the appropriate equations must be derived. To simplify the analysis, we will consider that the rapid distribution phase has been passed, and that MTQ has been extensively distributed from plasma to the two extravascular compartments, i.e., the equations derived will pertain only to data collected at times greater than about 10 hours after drug ingestion.

To obtain the kinetic equations, we will assume for simplicity that the concentration of free MTQ in plasma is essentially constant (steady-state approximation). Expressed mathematically, this approximation states that

$$\frac{d[MTQ_{Pf}]}{dt} = 0 = k_1[MTQ_{EV}] + k_{-2}[MTQ_U] - (k_2 + k_{-1})[MTQ_{Pf}]$$

$$[MTQ_{Pf}] = \frac{k_1[MTQ_{EV}] + k_2[MTQ_U]}{k_2 + k_{-1}} \qquad (3)$$

This is generally a very good approximation for reaction intermediates when (1) the intermediates are highly reactive and therefore are present at low concentrations, and (2) the initial induction period or lag time has been passed.[41] Both criteria appear to be satisfied here, (1) because the free plasma concentration is maintained low due to extensive binding to plasma proteins, and (2) because we are only considering time periods after the initial rapid distribution phase. An additional factor which tends to support the steady-state hypothesis for MTQ_{Pf} is "buffering" due to the rapidity and extent of plasma protein binding. A relatively large "sink" of MTQ is always present in the bound form in plasma, which tends to minimize changes in the concentration of free MTQ, analogous to the process whereby pH is maintained approximately constant in a system containing an acid-base buffer.

It should be understood that the assumption of a constant level of free MTQ in plasma does not imply that the <u>total</u> plasma concentration is constant. The total plasma concentration can and does change considerably due to changes in the MTQ levels of other tissues and compartments. The plasma concentration changes, however, should occur primarily in the level of <u>bound</u> MTQ, since this form constitutes the major plasma component. For example, consider a change, Δ_t, in the total MTQ concentration of plasma. Then it may be shown that the change in the <u>free</u> MTQ concentration of plasma, Δ_f, is given by

$$\Delta_f = \Delta_t \frac{K_P}{1 + K_P} \tag{4}$$

Since $K_P \cong 0.1$ in the therapeutic range,[40] the absolute change in the concentration of free MTQ_P will be much smaller than the absolute change in the total MTQ_P level. It is, therefore, a good approximation to consider that changes in the MTQ level of plasma occur essentially entirely in the bound fraction.

The differential rate equations describing the kinetics of MTQ_{EV} and MTQ_U are, respectively,

$$\begin{aligned} d[MTQ_{EV}]/dt &= -(k_1 + k_m)[MTQ_{EV}] + k_{-1}[MTQ_{Pf}] \\ d[MTQ_U]/dt &= k_2[MTQ_{Pf}] - k_{-2}[MTQ_U] \end{aligned} \tag{5}$$

Substitution of eq. 3 in eqs. 5 and rearrangement yields

$$\begin{aligned} d[MTQ_{EV}]/dt + (k_m + k_e)[MTQ_{EV}] - k_{-e}[MTQ_U] &= 0 \\ d[MTQ_U]/dt - k_e[MTQ_{EV}] + k_{-e}[MTQ_U] &= 0 \end{aligned} \tag{6}$$

where, by definition

$$k_e = \frac{k_1 k_2}{k_{-1} + k_2} \quad \text{and} \quad k_{-e} = \frac{k_{-1} k_{-2}}{k_{-1} + k_2} \tag{7}$$

Integration of these simultaneous differential equations can be performed by a variety of techniques. For the present, we will use a method outlined by Frost and Pearson.[42]

Assume particular solutions of the form

$$[MTQ_{EV}] = B_{EV} e^{-\lambda t} \quad \text{and} \quad [MTQ_U] = B_U e^{-\lambda t} \tag{8}$$

Substitution of eqs. 8 in eqs. 6 and cancellation of the exponential in every term leads to a pair of algebraic equations in the B_i's:

$$\begin{aligned} (k_e - \lambda)B_U - k_e B_{EV} &= 0 \\ -k_{-e} B_U + (k_m + k_e - \lambda)B_{EV} &= 0 \end{aligned} \tag{9}$$

Except for the trivial and physically uninteresting case that the B_i are equal to zero, eq. 9 can only be true if the determinant of the coefficients is equal to zero, i.e.,

$$\begin{vmatrix} k_e - \lambda & -k_e \\ -k_{-e} & k_m + k_e - \lambda \end{vmatrix} = 0 \qquad (10)$$

Solving for λ, we obtain the two characteristic roots,

$$\lambda_{1,2} = \frac{k_e + k_{-e} + k_m}{2} \pm \frac{[(k_e + k_{-e} + k_m)^2 - 4k_m k_{-e}]^{\frac{1}{2}}}{2} \qquad (11)$$

Corresponding to each λ_r, there will be a set of relative values of the B_i's that satisfy eqs. 9. Let these be denoted by B_{EVr} and B_{Ur}. Then, for any λ_r we have from eq. 9:

$$\begin{aligned} (k_e - \lambda_r)B_{Ur} - k_e B_{EVr} &= 0 \\ -k_{-e} B_{Ur} + (k_m + k_e - \lambda_r)B_{EVr} &= 0 \end{aligned} \qquad (12)$$

The relative values of B_i's can be obtained by making the arbitrary assumption that the two B_{EVr} values are equal to unity. Then we obtain from the first of eq. 12:

$$B_{Ur} = \frac{k_e}{k_{-e} - \lambda_r} \qquad (13)$$

The general solution of the simultaneous differential equation is obtained by writing a linear combination of particular solutions,

$$[MTQ_i] = \sum_{i=1}^{2} B_{ir} Q_r e^{-\lambda_r t} \qquad (14)$$

where the Q_r's are constants to be determined from the initial conditions. Specifically,

$$[MTQ_{EV}] = Q_1 e^{-\lambda_1 t} + Q_2 e^{-\lambda_2 t}$$

$$[MTQ_U] = \frac{k_e}{k_{-e} - \lambda_1} Q_1 e^{-\lambda_1 t} + \frac{k_e}{k_{-e} - \lambda_2} Q_2 e^{-\lambda_2 t} \tag{15}$$

Since these equations were derived for times subsequent to the initial rapid distribution phase, both $[MTQ_{EV}]$ and $[MTQ_U]$ will be nonzero at an arbitrary time t = 0 after the rapid distribution phase. We will call these "initial" values $[MTQ_{EV}^{o'}]$ and $[MTQ_U^{o'}]$ to signify that t = 0 for this derivation does not correspond to the actual zero time of the experiment.

By substitution in eqs. 15 we have

$$[MTQ_{EV}^{o'}] = Q_1 + Q_2$$

$$[MTQ_U^{o'}] = \frac{k_e}{k_{-e} - \lambda_1} Q_1 + \frac{k_e}{k_{-e} - \lambda_2} Q_2 \tag{16}$$

These two equations can be solved for the Q_r's, most easily by using Cramer's rule. The final equations that we obtain are of the form

$$[MTQ_{EV}] = \frac{1}{(\lambda_2 - \lambda_1)} [(k_{-e} - \lambda_1)A_1 e^{-\lambda_1 t} + (k_{-e} - \lambda_2)A_2 e^{-\lambda_2 t}] \tag{17}$$

$$[MTQ_U] = \frac{k_e}{(\lambda_2 - \lambda_1)} [A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t}] \tag{18}$$

where

$$A_1 = [MTQ_{EV}^{o'}] - \frac{(k_{-e} - \lambda_2)}{k_e} [MTQ_U^{o'}]$$

$$A_2 = \frac{(k_{-e} - \lambda_1)}{k_e} [MTQ_U^{o'}] - [MTQ_{EV}^{o'}] \tag{19}$$

The equation for $[MTQ_U]$ may be written in abbreviated form as

$$[MTQ_U] = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \tag{20}$$

where

$$C_i = \frac{k_e}{\lambda_2 - \lambda_1} A_i \tag{21}$$

The constants C_1, C_2, λ_1, and λ_2 in eq. 20 can be determined from the experimental data for times greater than 10 hr. A preliminary fit is illustrated in Figure 11, and the constants obtained are summarized in Table VIII. These constants have not been determined by least-squares, and it should be stressed that they represent preliminary estimates only. It is, however, of interest that the rate constant essentially characterizing the disappearance of MTQ between 10 and 50 hr, λ_1, is in the range of values reported by others[17,22] for the "terminal" β-phase according to the two-compartment model. Some simple relationships among these constants and the constants of the three-compartment model are:

$$\lambda_1 \lambda_2 = k_m k^{-e}$$

$$\lambda_1 + \lambda_2 = K_e + k_{-e} + k_m$$

$$C_1 \pm C_2 = [MTQ_U^{o'}]$$

$$C_1\lambda_1 + C_2\lambda_2 = k_{-e}[MTQ_U^{o'}] - k_e[MTQ_{EV}^{o'}]$$

It is seen that the number of experimental constants is insufficient to determine unique values for the various rate constants. We have, however, neglected the rapid distribution phase (2 hr < t < 10 hr), and with the addition of these data it will be possible to obtain estimates of all of the constants. Unfortunately, time does not permit a complete analysis to be performed for this report.

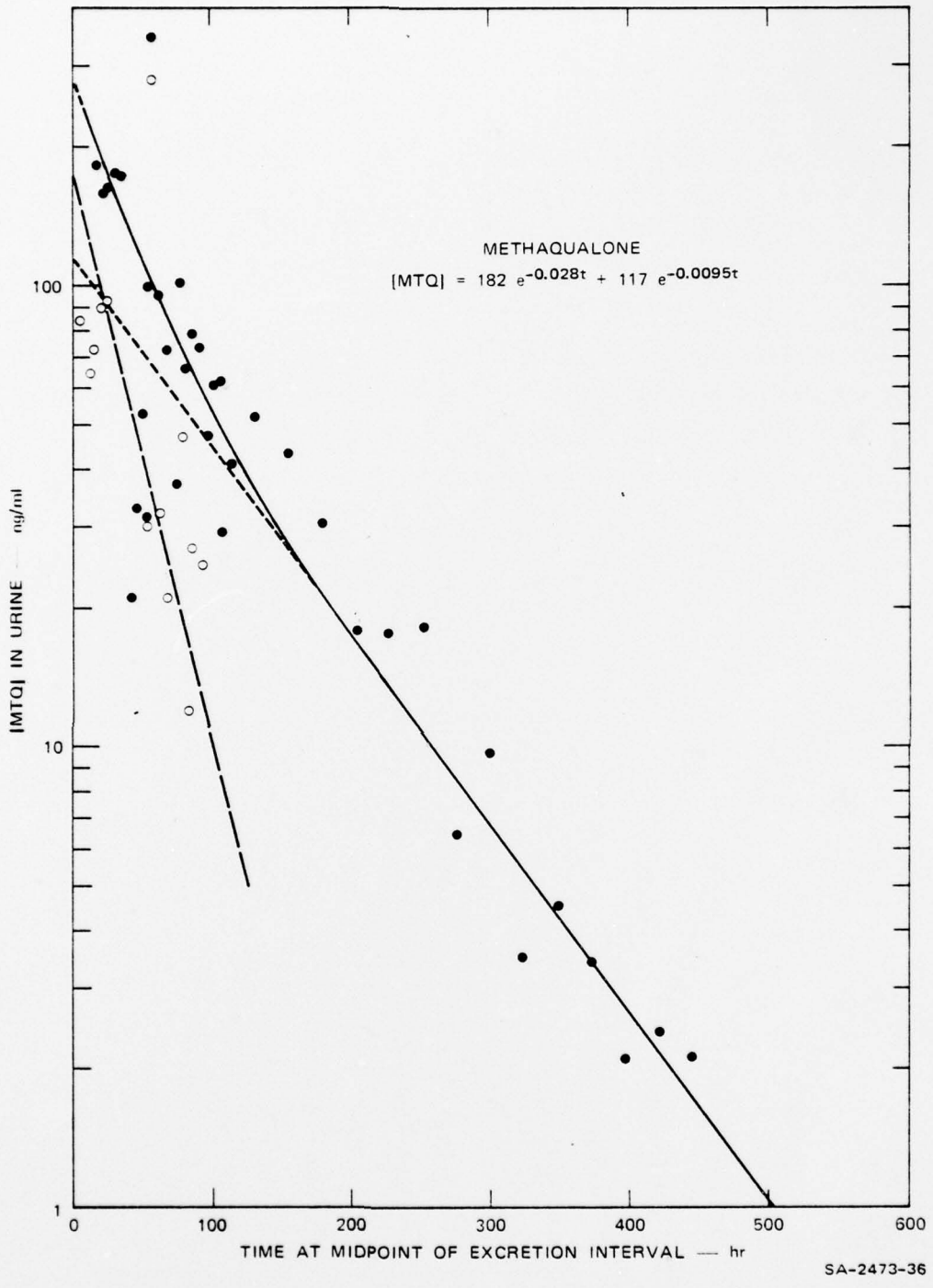


FIGURE 11 FIT OF METHAQUALONE EXCRETION DATA OBTAINED AFTER 10 HOURS TO A SUM OF TWO EXPONENTIALS

TABLE VIII. APPROXIMATE PHARMACOKINETIC CONSTANTS OF MTQ EXCRETION FOR TIMES GREATER THAN 10 HR

Constant	Estimated Value
C_1	182 ng/ml
C_2	117 ng/ml
λ_1	0.028 hr^{-1}
λ_2	0.0095 hr^{-1}

The important conclusion of the model is that, for times subsequent to the rapid distribution phase, it predicts that the urinary concentration will decrease in a biexponential manner, which appears to be in agreement with the experimental data. Moreover, the model does not appear to incorporate arbitrary or unusual pharmacological assumptions. It must, however, be realized that the model is not unique, and many other three-compartment open models could be fitted to the data. Similarly, models having four (or more) compartments could also be imagined, and would undoubtedly be closer to physiological reality than is Model 3. To exclude a three-compartment model, however, would require significantly less scatter in the data points. Thus, Model 3 may be regarded as a tentative hypothesis until such time as more precise pharmacokinetic data may be forthcoming. Whether or not the model is physiologically "correct," however, is less important than its eventual clinical usefulness for planning therapeutic regimens or its ability to provide insight into the long-term pharmacokinetics of MTQ.

The analyses of the pharmacokinetics of unconjugated MTQOH and total MTQOH can be performed similarly to that of MTQ, although we will not do so at this time. It will be of particular interest to interpret the early stages of excretion of these compounds, since information regarding the pharmacokinetics of drug metabolism should result from these data. Analyses of the complete pharmacokinetics of all these compounds are in progress, and will be published in due course.

VI CONCLUSIONS

MTQ was recently described as the "hypnotic of choice" on the basis of its apparently lower toxicity than the barbiturates.[43] Recent evidence has shown, however, that MTQ induces physical dependency in mice,[44] and other authors have suggested that it may do so in humans.[5] It appears that MTQ abuse has become widespread in recent years.[2-4]

In order to determine the extent to which drugs are being abused by individuals or in a population, it is necessary that drug concentrations be measured in bodily fluids, generally in urine. Our study has shown that if such a screening program were undertaken on a random basis using isotope dilution analysis and field ionization mass spectrometry, it would be possible to detect levels of MTQ or MTQOH in urine down to about 1 ng/ml. The pharmacokinetics of MTQ dictate the time range over which measurable concentrations can be found.

The 30-day study described herein together with the earlier 11-day study have shown that the terminal phase of excretion of MTQ is characterized by a half-life of 50 to 75 hr. When coupled with a sensitive analytical technique, this implies that MTQ or MTQOH can be determined in urine for as long as 30 days after ingestion of a single tablet.

MTQ is typical of a number of highly lipophilic drugs that are characterized by long half-lives in the body. Such drugs are poorly excreted and are extensively metabolized. We consider it quite probable that a study such as the present, if carried out on other slowly excreted drugs, such as the barbiturates, tricyclic antidepressants, ethanol, or opiates, would show that these drugs also are excreted with much longer half-lives than indicated by data obtained using less sensitive analytical procedures over relatively short time spans. It does not seem unlikely that a three-compartment open model such as that given in Section V would be able to

describe the pharmacokinetics of many such compounds.

In summary, field ionization mass spectrometry has been shown to be a powerful tool for determining low levels of drugs in bodily fluids. Although the sample measurement time is rather longer than the more commonly used procedures of tlc, gas chromatography, or gas chromatography/mass spectrometry, the sensitivity is correspondingly higher. The present study has provided a thorough test of our methodology when dealing with relatively large numbers of samples of unknown origin. The results obtained strongly support the further application of these techniques to problems in pharmacology and toxicology.

VII REFERENCES

1. McReynolds, J. H.; Heck, H. d'A.; Anbar, M. Biomed. Mass Spectrom. 1975, 2, 299.

2. Pascarelli, E. P. J. Amer. Med. Assoc. 1973, 224, 1512.

3. Inaba, D.S.; Gay, G.R.; Newmeyer, J.A.; Whitehead, C. J. Amer. Med. Assoc. 1973, 224, 1505.

4. Bailey, D.N.; Jatlow, P.I. Clin. Chem. 1973, 19, 615.

5. Smith, D.E.; Wesson, D.R. Annu. Rev. Pharmacol. 1974, 14, 513.

6. Akagi, M.; Oketani, Y.; Takada, M. Chem. Pharm. Bull. 1963, 11, 62.

7. Pirl, J.N.; Rotterman, V.M.; Fiorese, F.F. Anal. Chem. 1972, 44, 1675.

8. Brown, S.S. J. Pharm. Pharmacol. 1969, 21, 466.

9. Burnett, D.; Goudie, J.H.; Sherriff, J.M. J. Clin. Path. 1969, 22, 602.

10. Sleeman, H.K.; Cella, J.A.; Harvey, J.L.; Beach, D.J. Clin. Chem. 1975, 21, 76.

11. Berry, D.J. J. Chromatog. 1969, 42, 39.

12. Mitchard, M.; Williams, M.E., J. Chromatog. 1972, 72, 29.

13. Douglas, J.F.; Shahinian, S. J. Pharm. Sci. 1973, 62, 835.

14. Evenson, M.A.; Lensmeyer, G.L. Clin. Chem. 1974, 20, 249.

15. Bonnichsen, R.; Claes-Göran, F.; Negoita, C.; Ryhage, R. Clin. Chim. Acta 1972, 40, 309.

16. Bonnichsen, R.; Marde, Y.; Ryhage, R. Clin. Chem. 1974, 20, 230.

17. Alván, G.; Lindgren, J-E.; Bogentoft, C.; Ericsonn, Ö. Europ. J. Clin. Pharmacol. 1973, 6, 187.

18. Alván, G.; Ericsson, Ö.; Levander, S.; Lindgren, J.-E. Europ. J. Clin. Pharmacol. 1974, 7, 449.

19. Smyth, R.D.; Lee, J.K.; Polk, A.; Chemburkar, P.B.; Savacool, A.M. J. Clin. Pharmacol. 1973, 13, 391.

20. Morris, R.N.; Gunderson, G.A.; Babcock, S.W.; Zaroslinski, J.F. Clin. Pharmacol. Therap. 1972, 13, 719.

21. Polk, A.; DeLong, A.F.; Smyth, R.D.; Herczeg, T.; Burns, B.J.; Klunk, L.J.; Reavey-Cantwell, N.H. Drug Metab. Dispos. 1974, 2, 365.

22. Nayak, R.K.; Smyth, R.D.; Chamberlain, J.H.; Polk, A.; DeLong, A.F.; Herczeg, T.; Chemburkar, P.B.; Joslin, R.S.; Reavey-Cantwell, N.H. J. Pharmacokin. Biopharm. 1974, 2, 107.

23. Klosa, J. J. Prak. Chem. 1963, 20, 283.

24. Lawson, J.A., in preparation.

25. Anbar, M.; Aberth, W. Anal. Chem. 1974, 46, 59A.

26. Nowak, H.; Schorre, G; Struller, R. Arzneim.-Forsch. 1966, 16, 407.

27. Weissman, N.; Lowe, M.L.; Beattie, J.M.; Demetriou, J.A. Clin. Chem. 1971, 17, 875.

28. Osborne, D.N.; Gore, B.H. J. Chromatog. 1973, 77, 233.

29. Preuss, Fr. R.; Hassler, H.-M.; Köpf, R. Arzneim.-Forsch. 1966, 16, 395.

30. Miller, W. L.; Kulberg, M.P.; Banning, M.E.; Brown, L.D.; Doctor, B.P. Biochem. Med. 1973, 7, 145.

31. Wagner, J. G. J. Pharm. Sci. 1963, 52, 1097.

32. Wagner, J.G. Fundamentals of Clinical Pharmacokinetics 1975, p. 83, Drug Intelligence Publications, Hamilton, Ill.

33. Wagner, J.G. ibid., pp. 114-120.

34. Rescigno, A.; Segre, G. Drug and Tracer Kinetics 1966, pp. 66-68, Blaisdell, Waltham, Mass.

35. Rescigno, A.; Segre, G. ibid., pp. 92-96.

36. Rotblat, J.; Marcus, R. in Radioisotope Techniques 1953 Vol. 1, p. 33, Her Majesty's Stationery Office.

37. Freeman, T.; Matthews, C.M.E. in Radioaktive Isotope in Klinik and Forschung 1958 (von Fellinger, K.; Vetter, H., eds.), Vol. 3, p 283, Urban and Schwarzenberg, Munich.

38. Wagner, J. G. Fundamentals of Clinical Pharmacology, op. cit., pp. 38-42.

39. Brodie, B. B. in Absorption and Distribution of Drugs 1964, (Binns, T. B., ed.), p. 199, Williams and Wilkins, Baltimore.

40. Smart, G. A.; Brown, S. S. Anal. Biochem. 1970, 35, 518.

41. Frost, A. A.; Pearson, R. G. Kinetics and Mechanism 1961, 2nd edition, p. 172, John Wiley, New York.

42. Frost, A. A.; Pearson, R. G. ibid., pp. 173-177.

43. Suttell, R.; Cavaillé, G. Clin. Toxicol. 1975, 8, 359.

44. Alpern, H. P.' Greer, C. A.; Stripling, J. S.; Collins, A. C.; Olson, R. K. Psychopharmacologia 1975, 44, 303.

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